Reference: *Biol. Bull.* **178:** 210–216. (June, 1990)

Effect of Calcium on the Stability of the Vitelline Envelope of Surf Clam Oocytes

GEORGE DESSEV AND ROBERT GOLDMAN

Department of Cell, Molecular and Structural Biology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, Illinois 60611, and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Abstract. Fertilization and parthenogenic activation of oocytes of the surf clam, *Spisula solidissima*, require the presence of calcium in the extracellular medium. Here we report that the depletion of calcium causes a dramatic increase in the stability of the vitelline envelopes (VE). On the basis of this effect, we have developed a method of isolating intact VE and have studied their morphology, composition, and properties. Experiments using $^{45}Ca^{2+}$ have revealed that isolated VE bind calcium in a weak, but specific way. These findings suggest that the function of calcium may be to maintain the oocyte surface in a fertilization-competent state, while the reactions subsequent to the initial activation event, and leading to nuclear envelope breakdown (NEBD), may not require calcium. In support of this hypothesis, we have demonstrated that hypertonic conditions induce the oocytes to undergo NEBD in the absence of extracellular calcium.

Introduction

Development of the oocytes of most animal species is discontinued at a certain stage of meiosis, usually at the G_2/M border (Masui and Clarke, 1979; Maller and Krebs, 1980; Maller, 1985). The oocytes can remain in a state of arrest for long periods and then resume the meiotic process as a response to external signals. In most vertebrates, these signals are hormone-like substances (Maller and Krebs, 1980; Maller, 1985). In *Spisula*, progress into M-phase is induced by fertilization or by a number of physical or chemical stimuli, such as UV-light or changes in the ionic composition of the medium (Allen, 1953). Whatever the nature of these signals, one of their early biochemical effects is believed to be the activation of a pleiotropic enzymatic system, termed M-phase promoting factor (MPF) (Masui and Markert, 1971; Smith and Eckert, 1971; Wu and Gerhart, 1980; Arion *et al.*, 1988; Draetta *et al.*, 1989; Dunphy *et al.*, 1988; Gautier *et al.*, 1988; Lohka *et al.*, 1988; Murray and Kirschner, 1989). Activation of MPF is supposed to trigger pathways leading to different M-phase specific events (*i.e.*, NEBD, condensation of chromosomes, formation of the mitotic spindle, karyokinesis, etc.).

Fertilization of the oocytes of the surf clam requires the presence of calcium in the extracellular medium (Allen, 1953; Schuetz, 1975; Jaffe, 1983, 1985). The mechanism of action of calcium is not known. Calcium has been put forward as a cofactor of protein kinase C (Dube *et al.*, 1987; Eckberg *et al.*, 1987). Alternatively, generation of the activating signal may require an interaction of calcium with the surface of the oocyte, as shown for other species (Moreau *et al.*, 1976; Schroderet-Slatkine *et al.*, 1976).

The experiments described in this paper were initiated to distinguish between these two mechanisms. We have obtained evidence suggesting that calcium interacts with the oocyte surface. The depletion of calcium has a striking effect on the stability of the vitelline envelope (VE), an extracellular structure surrounding the oocyte and closely associated with the plasma membrane. This finding has allowed us to develop a method of isolating pure VE, and to characterize them by biochemical and ultrastructural methods. Further, we have demonstrated that the isolated VE possess weak calcium-specific binding sites. Our observations suggest that the role of calcium may be to maintain the *Spisula* oocyte surface in a fertilization-competent state, rather than to participate as a co-

Abbreviations: VE, vitelline envelopes; NEBD, nuclear envelope breakdown; MFSW, millipore-filtered seawater; CFSW, calcium-free seawater; MPF, M-phase promoting factor.

factor in the enzymatic pathway leading to NEBD. In agreement with this view, we have demonstrated that treatments with hypertonic solutions of NaCl or glycerol induce the oocytes to resume maturation in the absence of extracellular calcium.

Materials and Methods

Surf clams were supplied by the Department of Marine Resources at the Marine Biological Laboratory (Woods Hole, Massachusetts). The oocytes were obtained by dissection of the ovaries of female animals and were then washed four times with Millipore-filtered seawater (MFSW) (Allen, 1953).

The proportion of oocytes that had undergone NEBD was scored by phase contrast microscopy. For electron microscopy, pellets of isolated VE were fixed in 1% glutaraldehyde in phosphate-buffered saline (pH 7.5), post-fixed in OsO_4, embedded in Epon, and sectioned. Proteins were fractionated by SDS PAGE (Laemmli, 1970). Protein was determined by the method of Lowry *et al.* (1951), and carbohydrates by the phenol/sulfuric acid method, as described by Ashwell (1966).

The binding of $^{45}Ca^{2+}$ to isolated VE was studied as follows. The VE were washed 5 times, each time in 200 volumes of binding buffer (0.25 *M* sucrose, 10 m*M* Pipes·NaOH, pH 7.2, 5 m*M* KCl, and 1 mg/ml bovine serum albumin), and resuspended in binding buffer at a protein concentration of 200–250 μg/ml. Aliquots of this suspension were incubated for 30 min at room temperature in the presence of $[^{45}Ca]CaCl_2$ (New England Nuclear; specific radioactivity 20 mCi/mg) at different concentrations of $CaCl_2$. At the end of the incubation, aliquots were taken for determination of the total radioactivity. The samples were then centrifuged in an Eppendorf microfuge for 5 min, and a second set of aliquots was taken from the supernatant to determine the unbound radioactivity. The binding was calculated from the difference between the two measurements. The walls of the tubes adsorbed less than 3% of the radioactivity. The presence of bovine serum albumin, which was necessary to minimize the adhesion of the VE to the plastic, did not affect the results. At all Ca^{2+} concentrations studied, 30 min of incubation was sufficient to reach a constant ratio between free and bound calcium.

Results

Depletion of calcium leads to stabilization of the oocyte VE

In our first experiments, we studied the stability of the VE in the presence or absence of calcium. Oocytes were extensively washed with MFSW or calcium-free seawater (CFSW) and then vigorously homogenized in an all glass homogenizer. After 20 strokes by hand, more than 90% of the oocytes suspended in MFSW were broken and their VE were extensively fragmented. After the same treatment, only about 30% of the oocytes in CFSW were broken and, more important, the VE of the broken cells were nearly intact empty shells. These results suggested that the presence of calcium strongly affects the mechanical stability of the VE.

Table I

Effect of different treatments on the stability of the vitelline envelopes of surf clam oocytes

Treatment of oocytes	State of the VE
10% glycerol, 15 m*M* phosphate buffer, pH 8, 0.5% NP-40	Solubilized in 10 min at 20°C
10% glycerol, 15 m*M* phosphate buffer, 0.5% NP-40, and 20 m*M* EGTA	No lysis
SDS, 0.2%	Solubilized in 10 min at 20°C
SDS, 0.2% in 20 m*M* EGTA	No lysis
4 *M* urea	Solubilized in 10 min at 20°C
4 *M* urea in 20 m*M* EGTA	No lysis

Further experiments revealed that a similar effect could be observed upon treating the oocytes with reagents disrupting protein-protein interactions, such as 4 *M* urea, 0.2% SDS, and with a solution containing 1.4 *M* glycerol, 15 m*M* phosphate, pH 8.0, (with or without 50 m*M* KCl), which destabilizes the VE (Rebhun and Sharpless, 1964; Dessev and Goldman, 1988), followed by 0.5% NP-40. In all these cases, if the samples contained 1–10 m*M* $CaCl_2$, the oocytes lysed and nearly all of the VE were dissolved, as observed by phase contrast optics (Table I). In contrast, when the samples contained 5–10 m*M* EGTA, the cells did not lyse and the integrity of the VE did not seem to be affected (Table I). The EGTA-induced stabilization of the VE was reversible: after addition of $CaCl_2$ to an excess of 10 m*M*, the VE underwent fragmentation over a period of 10–20 min and finally dissolved, which was accompanied by lysis of the oocytes. EGTA caused stabilization of VE when added both before and after the oocytes had undergone NEBD following KCl activation.

These results demonstrate that calcium strongly affects the mechano-chemical properties of the VM: in the presence of EGTA, a marked stabilization of these structures occurs, rendering them resistant to conditions which would completely solubilize them in the presence of calcium.

Isolation of VE

The EGTA-induced stabilization of the VE allowed us to develop the following procedure for their isolation.

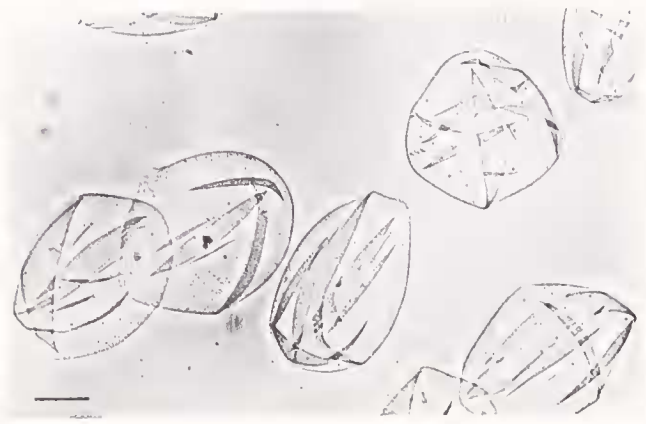

Figure 1. VE isolated as described in the text. Phase contrast. Bar, 20 μm.

Oocytes were sedimented by gravity from 500 volumes of CFSW containing 10 m*M* EGTA, and washed two times with 50 volumes of a solution containing 40 m*M* PIPES (pH 7.2), 100 m*M* NaCl, 10 m*M* KCl, 5 m*M* $MgCl_2$, 50 m*M* EGTA, and 10 m*M* dithiothreitol. The cells were then vigorously homogenized with an all-glass homogenizer in 20 volumes of the same solution at 0°C. The homogenization was continued, with intermittent microscopic control, until virtually all cells were lysed. The homogenate contained empty VE, cytoplasmic granules, and small nuclear fragments. The sample was centrifuged for 10 min at 2000 r.p.m. in a TJ-6 Beckman centrifuge. The crude VE pellet was resuspended in the same solution containing 0.25 *M* sucrose and 0.5% NP-40, and the structures were sedimented again at 2000 r.p.m. The latter step was repeated two more times, yielding a preparation of pure VE (Figs. 1, 2).

If the same procedure was performed on oocytes washed with MFSW, and if the homogenization solution contained 1 m*M* $CaCl_2$ instead of EGTA, then the VE were broken into small fragments, which were impossible to purify.

Ultrastructure

Electron micrographs of isolated VE reveal a structure generally similar to that observed earlier by others (Rebhun, 1962; Longo and Anderson, 1970) on sections of whole oocytes (Fig. 3). The structures consist of a continuous layer of amorphous material, 40–80 nm thick, surrounding the remnants of the microvilli, or spaces formerly occupied by microvilli, located in approximately hexagonal arrays. The inner surface of the VE is associated with smooth sheets or oval-shaped vesicles, which may originate from the oocyte plasma membrane. These sheets, as well as the remnants of the microvilli, probably represent residual, non-lipid, "skeletal" structure of the plasma membrane, as suggested by their resistance to the extensive washing with NP-40.

Our micrographs also reveal an outermost filamentous layer, located above the microvilli, perhaps originating from the oocyte jelly coat. This layer appears less electron-dense than the material surrounding the microvilli. It is separated from their tips by a narrow space which contains a large number of spherical bodies. These bodies are uniform in size (35–40 nm in diameter) and appear to have smooth envelopes.

Composition and properties

The isolated VE contain 55% protein and 45% carbohydrate. They show a complex SDS PAGE pattern (Fig. 4). A treatment of the VE with proteinase K (1 μg/μl at room temperature) leads to their dispersal in less than 1 min, suggesting that protein components are important for their structural integrity. Upon exposure to distilled water, the VE expand up to 50% in diameter. The honeycomb-like structure of the expanded VE can be clearly observed in phase contrast (Fig. 2). Purified VE can be kept frozen at −80°C, which does not affect their morphology or other properties.

Binding of $^{45}Ca^{2+}$ to isolated VE

The stabilizing effect of EGTA described above suggested a direct interaction between the VE and calcium. To verify this, we studied the binding of $^{45}Ca^{2+}$ to isolated VE. Our results showed that at each concentration of Ca^{2+} an equilibrium between free and VE-bound calcium was established, such that between 60% and 70%

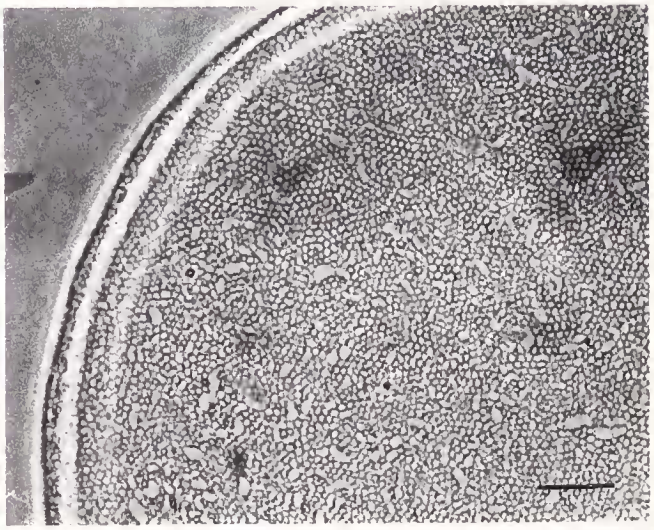

Figure 2. VE isolated as described in the text and resuspended in distilled water for 10 min at room temperature. Phase contrast. Bar, 5 μm.

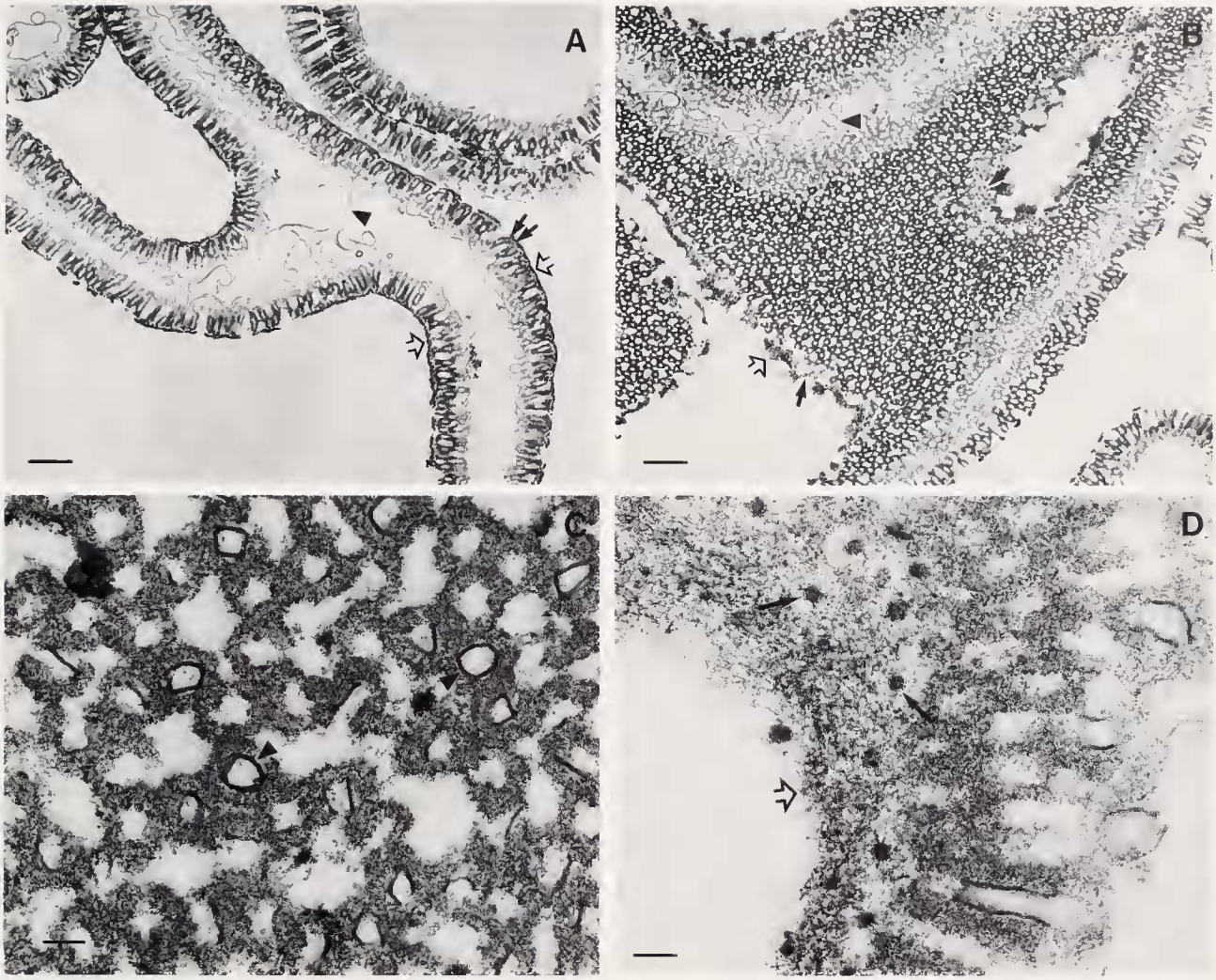

Figure 3. Electron micrographs of sections of isolated VE. (A, B), Bar, 1 μm; (C, D), Bar, 100 nm. A continuous layer of amorphous material, 40–80 nm thick, surrounds the remnants of the microvilli, or spaces formerly occupied by microvilli, located in approximately hexagonal arrays. The inner surface of the VE is associated with smooth sheets or oval-shaped vesicles (arrowheads), which also deliniate the microvilli. A filamentous layer located above the microvilli is seen (open arrows). It is separated from their tips by a narrow space, which contains a large number of spherical bodies, 35–40 nm in diameter, surrounded by smooth envelopes (solid arrows).

of the input calcium cosedimented with the VE at concentrations of Ca^{2+} below 10 μM (Fig. 5). Practically all of the bound radioactivity could be removed by several washings of the pellet with binding buffer. As the concentration of Ca^{2+} increased, the ratio bound Ca^{2+}/input Ca^{2+} declined, and the binding reached an apparent saturation between 10 mM and 40 mM Ca^{2+} at approximately 6 μg bound Ca^{2+} per mg VE-protein (Fig. 6). Despite the low affinity of the VE for Ca^{2+}, the binding was specific, as demonstrated by Mg^{2+} competition experiments (Fig. 7). A 1000-fold excess of Mg^{2+} suppressed the binding of $^{45}Ca^{2+}$ by less than 50%; under the same conditions a much stronger competition by unlabeled calcium was observed (Fig. 7).

Hypertonic conditions induce oocyte activation in the absence of extracellular calcium

Although our results suggested that the action of calcium is restricted to the oocyte surface, they did not preclude the possibility that the stabilization of the VE and the inhibition of oocyte activation are two independent effects of calcium depletion. To obtain more evidence concerning this possibility, we studied the dependence of

Figure 4. SDS PAGE of isolated VE in 7.5% gel. The positions of the molecular weight markers are shown.

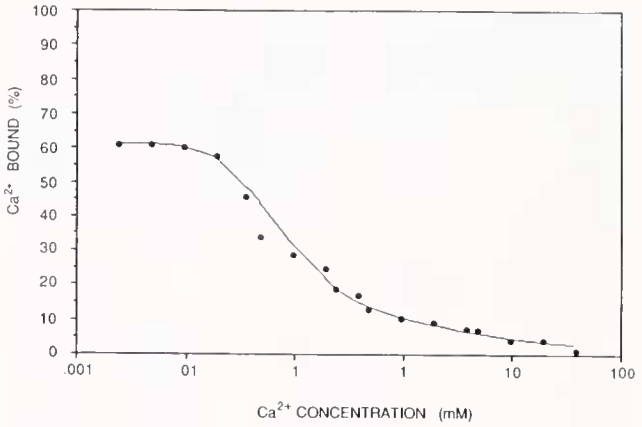

Figure 6. Binding of $^{45}Ca^{2+}$ to isolated VE was determined under the same conditions as in Figure 5, but at Ca^{2+} concentrations up to 40 m*M*

oocyte activation on calcium, using hypertonic solutions (Allen, 1953) such as 1.4 *M* glycerol and 1.2 *M* NaCl as activating agents. The rationale for these experiments was the following.The initial events that induce the oocytes to resume maturation are believed to involve cell depolarization caused by changes in permeability (Finkel and Wolf, 1978; Jaffe, 1983, 1985). Our experiments suggested that calcium, by interacting with the oocyte surface, maintains the latter in a structural state necessary for these events to occur. We hypothesized that unphysiological treatments, such as osmotic shock, might disturb the permeability barrier at the oocyte surface and lead to depolarization and activation in the absence of calcium. Furthermore, glycerol, known to "soften" the VE (Rebhun and Sharpless, 1964), might be expected to counteract the EGTA-induced stabilization which was thought to prevent the oocyte activation.

We found that the oocytes could be activated in CFSW containing glycerol and 50 m*M* KCl. The proportion of activated oocytes did not change linearly with glycerol concentration, but increased abruptly around 10% (1.4 *M*) glycerol, suggesting a correlation between activation and hypertonicity (Fig. 8). No activation occurred in CFSW and 50 m*M* KCl alone. Activation (30–50%) was also observed in solutions containing 1.4 *M* glycerol, 20 m*M* Na-phosphate (pH 8.0), 50 m*M* KCl, and 30–80 m*M* EGTA (not shown).

In another series of experiments, we used a procedure involving concentrated solutions of NaCl to activate the cells (Allen, 1953). Oocytes were extensively washed in CFSW and resuspended in a mixture of CFSW and 0.2 *M* EGTA (1:1). To this sample, 0.25 volumes of 5 *M* NaCl were added to a final NaCl concentration of 1.2 *M*. After 15 min at 18°C, the cells were sedimented and

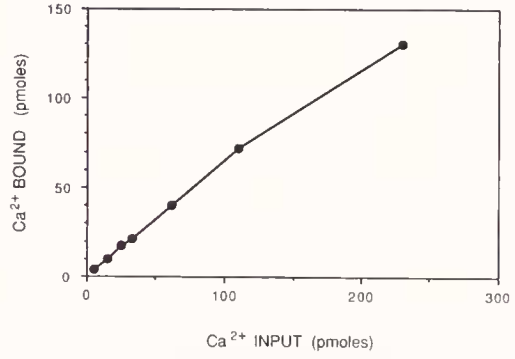

Figure 5. Binding of $^{45}Ca^{2+}$ to isolated VE at low concentrations of Ca^{2+}. VE suspended in binding buffer at a protein concentration of 250 μg/ml were incubated with different amounts of $^{45}Ca^{2+}$ in a final volume of 0.1 ml. The binding was determined as described in Materials and Methods.

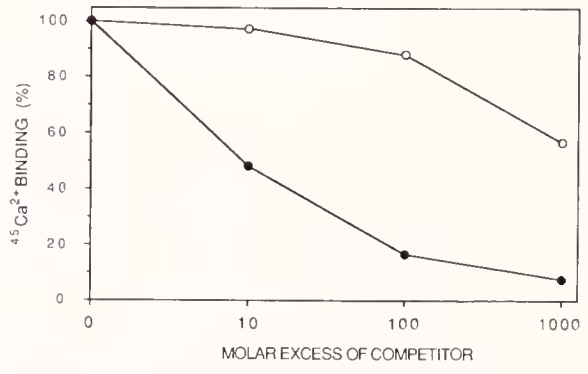

Figure 7. Binding of $^{45}Ca^{2+}$ (initial concentration 0.5 μ*M*) to isolated VE in the presence of increasing concentrations of Mg^{2+} (open symbols) and Ca^{2+} (closed symbols).

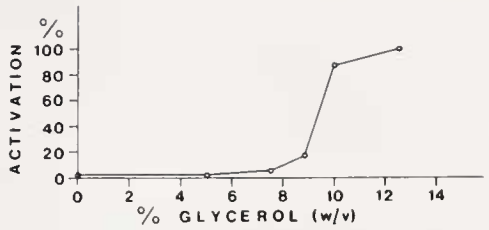

Figure 8. Activation of oocytes in glycerol solutions. Oocytes were suspended in CFSW containing 50 m*M* KCl and glycerol at the indicated concentration. Thirty minutes later, the percentage of oocytes that had undergone NEBD was scored.

resuspended in the same solution without NaCl. Twenty minutes later, the proportion of oocytes that had undergone NEBD was determined. The results varied between 40% and 80% activation in different experiments.

These studies demonstrate that under hypertonic conditions the oocytes can be activated in the absence of extracellular calcium.

Discussion

Our results suggest that calcium interacts with the VE of the surf clam oocytes and that this interaction may be important for maintaining the oocyte surface in a fertilization-competent state. This conclusion is based on the correlation between our experimental findings and the biological properties of the system. Thus, (a) depletion of calcium inhibits fertilization and causes a marked increase in the stability of the VE. Both effects are reversible upon addition of calcium. (b) The weak binding of calcium to isolated VE correlates with the seemingly weak binding of biologically important calcium; a brief washing with a calcium-free medium prevents fertilization or activation of the oocytes. (c) Mg^{2+} does not substitute for calcium in the process of activation, nor does it compete significantly with $^{45}Ca^{2+}$ binding *in vitro*.

A mechanism of action of calcium involving an interaction with the oocyte surface is consistent with studies on other species. Thus, introduction of calcium into zones near the surface of *Xenopus* oocytes leads to their activation, whereas calcium introduced deeper into the oocyte interior has no such effect (Moreau *et al.*, 1976). Similarly, treatment of the *Xenopus* oocyte surface with lanthanum (considered a calcium-mobilizing agent), but not its microinjection into the oocyte, induces maturation (Schorderet-Slatkine *et al.*, 1976).

The effect of phorbol esters on oocyte activation suggests that calcium- and phospholipid-dependent protein kinase C may be involved in the initial stages of maturation (Dube *et al.*, 1987; Eckberg *et al.*, 1987). Schuetz (1975) has concluded that, in the clam, calcium is rapidly equilibrated across the oocyte membrane, and the presence of calcium in the medium is needed to supply intracellular calcium. However, this does not seem to be the case, because hypertonic conditions cause oocyte activation in the presence of EGTA.

These results can be explained in two ways. Either (a) the hypertonic treatment releases calcium from EGTA-inaccessible intracellular sources, or (b) the reactions leading to NEBD do not require calcium. Although we cannot, at present, distinguish between these two explanations, the second one agrees with our finding that NEBD in a *Spisula* oocyte cell-free system does not require calcium (Dessev *et al.*, 1989). Moreover, cytosolic extracts from unstimulated *Spisula* oocytes, which are initially inactive in NEBD *in vitro*, can be activated in the absence of calcium (Dessev, unpub. results). Similarly, the MPF system, which is considered as an universal M-phase regulator, appears to function in a calcium-independent way and is stabilized by EGTA (Wu and Gerhart, 1980; Arion *et al.*, 1988; Dunphy and Newport, 1988; Lohka *et al.*, 1988).

Activation factors, such as fertilization, UV light, and KCl require extracellular calcium (Allen, 1953). We have shown that other agents, such as hypertonic solutions of glycerol and NaCl, are capable of inducing maturation in the absence of calcium. Since glycerol and NaCl are chemically different, they are likely to act via osmotic changes, forcing membrane depolarization to occur even when the surface is structurally altered by depletion of calcium and is not susceptible to the action of other activation factors.

The chemical nature of the calcium binding to the VE, which is both weak and specific, as well as the nature of the structural and functional changes in the VE induced by calcium, remain interesting subjects for further studies.

Acknowledgments

We wish to thank Dr. A. Telser for help in the preparation of the drawings. Research support was provided by NCI.

Literature Cited

Allen, R. D. 1953. Fertilization and artificial activation in the egg of the surf clam, *Spisula solidissima*. *Biol. Bull.* **105:** 213–239.

Arion, D., L. Meijer, L. Brizuella, and D. Beach. 1988. cdc2 is a component of the M phase-specific histone H1 kinase: evidence for identity with MPF. *Cell* **55:** 371–378.

Ashwell, G. 1966. New colorimetric methods for sugar analysis. *Meth. Enzymol.* **8:** 85–95.

Dessev, G., and R. Goldman. 1988. Meiotic breakdown of nuclear envelope in oocytes of *Spisula solidissima* involves phosphorylation and release of nuclear lamin. *Dev. Biol.* **130:** 543–550.

Dessev, G., R. Palazzo, L. Rebhun, and Goldman R. 1989. Disassembly of the nuclear envelope of *Spisula* oocytes in a cell-free system. *Dev. Biol.* **313:** 496–504.

Draetta, G., F. Luca, J. Westendorf, L. Brizuella, J. Ruderman, and D. Beach. 1989. cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. *Cell* **56:** 829–838.

Dube, F., R. Golsteyn, and L. Dufresne. 1987. Protein kinase and meiotic maturation of surf clam oocytes. *Biochem. Biophys. Res. Comm.* **142:** 1072–1076.

Dunphy, W. G., L. Brizuella, D. Beach, and J. Newport. 1988. The *Xenopus* cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell* **54:** 423–431.

Dunphy, W., and J. W. Newport. 1988. Mitosis-inducing factors are present in a latent form during interphase in the *Xenopus* embryo. *J. Cell Biol.* **106:** 2048–2056.

Eckberg, W. R. 1983. The effect of quercetin on meiosis initiation in clam and starfish oocytes. *Cell Differ.* **12:** 329–334.

Eckberg, W. R., E. Z., Szuts, and A. G. Carrol. 1987. Protein kinase C activity, protein phosphorylation and germinal vesicle breakdown in *Spisula* oocytes. *Dev. Biol.* **124:** 57–64.

Finkel, T., and D. Wolf. 1978. Fertilization of surf clam oocytes: the role of membrane potential and internal pH. *Biol. Bull.* **155:** 437.

Gautier, J., C. Norbury, M. Lohka, P. Nurse, and J. Maller. 1988. Purified maturation-promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene cdc^{2+}. *Cell* **54:** 433–439.

Jaffe, L. F. 1983. Source of calcium in egg activation: a review and a hypothesis. *Dev. Biol.* **99:** 265–276.

Jaffe, L. F. 1985. The role of calcium explosions, waves and pulses in activating eggs. Pp. 127–165 in *Biology of Fertilization,* Vol. 2, C. B. Metz and A. Monroy, eds. Academic Press, New York.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

Lohka, M. J., M. K. Hayes, and J. L. Maller. 1988. Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proc. Natl. Acad. Sci. USA* **85:** 3009–3013.

Longo, F. J., and E. Anderson. 1970. An ultrastructural analysis of fertilization in surf clam, *Spisula solidissima* I. Polar body formation and development of female pronucleus. *J. Ultrastr. Res.* **33:** 495–514.

Lowry, O. M., N. J. Rosebrough, A. R. Farr, and R. G. Randall. 1951. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

Maller, J. L. 1985. Regulation of amphibian oocyte maturation. *Cell Differ.* **16:** 211–221.

Maller, J. L., and E. G. Krebs. 1980. Regulation of oocyte maturation. *Curr. Top. Cell Reg.* **16:** 217–311.

Masui, Y., and H. J. Clarke. 1979. Oocyte maturation. *Int. Rev. Cytol.* **57:** 185–282.

Masui, Y., and C. L. Markert. 1971. Cytoplasmic control of nuclear behaviour during meiotic maturation of frog oocytes. *J. Exp. Zool.* **177:** 349–356.

Moreau, M., M. Doree, and P. Guerrier. 1976. Electrophoretic introduction of calcium ions into the cortex of *Xenopus laevis* oocytes triggers meiosis reinitiation. *J. Exp. Zool.* **197:** 443–449.

Murray, A. W., and M. W. Kirschner. 1989. Dominoes and clocks: the union of two views of the cell cycle. *Science* **246:** 614–621.

Rebhun, L. I. 1962. Electron microscope studies on the vitelline membrane of the surf clam, *Spisula solidissima*. *J. Ultrastr. Res.* **6:** 107–122.

Rebhun, L., and T. K. Sharples. 1964. Isolation of spindles from the surf clam, *Spisula solidissima*. *J. Cell Biol.* **22:** 488–496.

Schorderet-Slatkine, S., M. Schorderet, and E. E. Baulieu. 1976. Initiation of meiotic maturation in *Xenopus laevis* oocytes by lanthanum. *Nature* **262:** 289–290.

Schuetz, A. W. 1975. Induction of nuclear breakdown and meiosis in *Spisula solidissima* oocytes by calcium ionophore. *J. Exp. Zool.* **191:** 443–446.

Smith, L. D., and R. E. Ecker. 1971. The interaction of steroids with *Rana pipiens* oocytes in the induction of maturation. *Dev. Biol.* **25:** 233–247.

Wu, M., and J. C. Gerhart. 1980. Partial purification and characterization of the maturation-promoting factor from eggs of *Xenopus laevis*. *Dev. Biol.* **79:** 465–477.